

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of : Gerard M. Housey, M.D., Ph.D.
Serial No. : 08/473,169
Filed : June 7, 1995
For : METHOD OF SCREENING FOR PROTEIN INHIBITORS AND
ACTIVATORS
Atty. Docket No. : 395-30
Examiner : Bonnie D. Weiss, Ph.D.
Art Unit : 1805

DECLARATION OF GERARD M. HOUSEY, M.D., Ph.D. UNDER 37 C.F.R. § 1.132

I, Gerard M. Housey, M.D., Ph.D., hereby depose and state that:

1. I am the named inventor on the above-captioned United States patent application.
2. I received a Bachelor of Science degree with majors both in Biochemistry and in Cellular and Molecular Biology, from the University of Michigan in 1981. I received a Doctorate (Ph.D.) in Genetics and Development from Columbia University in 1988. I received a medical degree (M.D.) from Columbia University's College of Physicians and Surgeons in 1991.
3. I have been performing research in the general areas of biochemistry and genetics since 1979, and, in particular, in the area of cellular signal transduction, initiation, promotion and progression of human solid tumors, as well as in pharmaceuticals development and discovery, out of which research I developed the invention embodied in the above-captioned patent application. I have also performed post-doctoral anti-cancer drug research at the National Cancer Institute, of the National Institutes of Health.

4. In the context of, for example, pharmaceutical research, there is a great deal of interest in investigating the interactions between certain substances and target proteins or other biologically significant species in order to assess the potential of the substances to act in a pharmaceutically effective manner. A cell capable of utilization in a method for screening test substances for their ability to inhibit or activate target proteins, such as that disclosed and claimed in my issued United States Patents No. 4,980,281 would, therefore, possess great utility.

5. I have read United States Patent No. 4,981,790 to Haseltine *et al.*, (the "Haseltine reference") and am familiar with both its teachings and the underlying research published in the scientific literature by both the inventors of the Haseltine reference and others in the field.

6. The Haseltine reference describes an invention comprising mammalian cell lines expressing the *tat_{III}* gene, a gene which is found in the HIV genome¹. The *tat_{III}* gene product (the *tat_{III}* protein) is known to bind to the HIV Long Terminal Repeat (LTR) sequence, and to enhance transcription from this region (also referred to as "transactivation"), as shown previously by the inventors of the Haseltine reference and others working in the field. In addition, under appropriate conditions, genes located downstream from the HIV LTR will be transcribed at an increased rate in the presence of high levels of *tat_{III}* protein.

7. The Haseltine reference suggests a variety of putative utilities derived from the availability of this cell line, including use as a tool for screening compounds that may have anti-HTLV-III/LAV activity. This specific utility is described in detail in Column 2, lines 20-33

¹Declarant notes that HTLV-III/LAV encompass older terms within the literature for what is now termed the Human Immunodeficiency Virus (HIV), and that various strains of HIV have subsequently been shown to exist.

(as also cited in the Office Action), as well as in Column 12, line 20 through Column 13, line 43 of the Haseltine reference.

8. In characterizing the teachings of the Haseltine reference, Declarant wishes to point out the distinctions between those teachings and the generalized methodology as taught in, for example, Declarant's United States Patent No. 4,980,281 and soon-to-issue patent from Application Serial No. 08/408,443, as well as in the disclosure of the instant application (collectively, the "Housey patents"). In general terms, Declarant wishes to direct the Examiner's attention to the extensive disclosures in those patents and applications, and to the fact that these disclosures describe, *for the first time*, the ability to specifically engineer a cell to become responsive to activators or inhibitors of *any* given target protein of interest.

9. For comparison purposes, the disclosures of the Housey patents include descriptions of generalized methods for creating or utilizing cells which possess a higher (usually non-naturally occurring) level of a "target" protein. Declarant was the first to demonstrate that an increase in the level of a given target protein in or on the surface of a cell results in the generation (*solely from the increased level of the protein in the cell*) of a steady state thermodynamic alteration in cellular behavior that gives rise to phenotypic changes which are responsive to inhibitors or activators of the target protein.

10. In accord with the Housey patents' definition and teachings, at least one *responsive* change in a phenotypic characteristic of the cell will result under the condition of an enhanced level of the target protein. Such a particular phenotypic change is, by definition, a "*responsive* change in a phenotypic characteristic" or "phenotypic response" because it exhibits the property of being responsive to substances which activate or inhibit the protein of interest. At least one (and frequently several) phenotypic change(s) will occur which is (are) test substance-responsive.

11. This core enabling teaching provides a simple yet highly effective way for an investigator skilled in the art to create a cell-based assay system useful for the identification of inhibitors or activators of virtually any type of protein, ***regardless of whether or not the investigator has any prior knowledge of the biological function of a given protein of interest.*** The sole selection criterion is that an enhanced level of the protein of interest within a cell be capable of inducing a phenotypic response in the cell, where that response can be further modulated by activation or inhibition of the protein of interest. In this system, it is totally unnecessary for the investigator to elucidate either the function(s) within the cell of the protein of interest, or the mechanism(s) of its inhibition or activation, prior to the use of the disclosed assay system.

12. By way of specific illustration from the Haseltine reference, the specification, at Col. 2, lines 57 - 64, indicates that the role of the tat_{III} protein in the prolific replication of the HIV has been elucidated as involving transactivation of the long terminal repeat sequence (LTR) of the HIV. Furthermore, as the specification of the Haseltine reference points out, and as stated in greater detail in references to the scientific literature cited in that specification (see, for example, Sodroski *et al.*, *Science* **227**:171 (1985)), the mechanism of action of the tat_{III} protein involves the essential step of binding of the protein to appropriate sites on the LTR. Thus, as discussed at great length in the specification of the Haseltine reference at Col. 12, line 21 to Col. 13, line 43, the assay system of the Haseltine reference cited by the Examiner in the outstanding Office Action is preferably directed toward screening for substances that somehow interfere with this essential binding step, perhaps by competitive binding. ("It is preferable to screen compounds that prevent the interaction of the tat_{III} protein with the sequences responsive to the tat_{III} protein in the HTLV-III LTR or prevent the ability of the tat_{III} protein to transactivate the HTLV-III LTR." Col. 12, lines 43 - 47.) Indeed, as

discussed in more detail below, the assay method of the Haseltine reference effectively possesses no concrete utility, according to the objective teachings of the specification, other than as a method of screening a number of mutational clones of the tat_{III} protein for their ability to competitively bind to the HIV LTR. The important conclusion that should be reached by one of skill in the appropriate art, based upon the teachings of the Haseltine reference, is that *without prior knowledge of the function of the tat_{III} protein and its mechanism of biochemical activity* there could be no assay at all. Elucidation of such function and mechanism is a non-trivial exercise, as can be borne out by an inspection of the relevant scientific literature. However, such prior knowledge of the function and mechanism of action of a target protein is not needed in the practice of the methods taught in the Housey patents.

13. In contrast, practice of the teachings of the Housey patents gives rise to a simple and straightforward method of determining whether a substance is an inhibitor or activator of a given target protein. One may simply determine whether a given substance is an activator or an inhibitor of the target protein by comparing changes in a particular phenotypic response, induced in the cell by the over-presence of the protein of interest, in the presence of a test substance, with respect to a control cell that lacks the responsive phenotypic change, the comparison being based on either contemporaneous observation or previously noted behavior of the control cell.

14. Substances so identified or characterized using the teachings of the Housey patents will be found to interact directly with the target protein itself and/or with other proteins in which the target protein also interacts in the form of dimeric or multimeric complexes. In this fashion, the approach disclosed in the Housey patents provides a *specificity* between a target protein and its attendant biochemical pathways within the cell that is impossible to achieve with other assay systems. This pioneering approach literally

created the concept of using whole cells as general biochemical assay tools for individual protein targets, thus freeing investigators from the procedural overhead typical of cell-free assay systems and, at the same time, providing a biochemical pathway specificity that significantly enhances the assay's correlation to *in vivo* results.

15. Turning to the teachings of the Haseltine reference in detail, the assay system described therein has an intended utility in the search for compounds which exhibit activity that interferes with the trans-activation of the tat_{III} gene product by *any* mechanism, whether or not the putative compound actually interacts with tat_{III} directly. In this respect, the disclosed assay system is designed to identify substances that "mitigate the cytopathic effects of the HTLV-III/LAV virus" (column 12, lines 21-24), *e.g.*, anti-HIV substances. Declarant notes that no such demonstration of the ability to utilize the assay to identify any known or novel substance with such activity is actually described or exemplified in the Haseltine reference. Nevertheless, this assay method does not, and cannot, provide the target protein/biochemical pathway specificity of the Declarant's invention as disclosed in the Housey patents.

16. If a given substance were to exhibit antiviral or anti-tat_{III} activity in the system of the Haseltine reference, as conceived by its inventors, it would be manifest by an alteration in the level of a "selectable" gene product (protein), such as the CAT protein, whose level would presumably be reduced in the presence of a compound which may inhibit tat_{III} function. The lack of specificity of such an assay method is apparent from the fact that such a finding may also result from a compound which interacts with numerous other viral or cellular proteins besides tat_{III}.

17. To further illustrate this last point, the disclosure of the Haseltine reference points out that it would be preferable to test substances that are **already known**, through prior

work, to be competitive inhibitors with respect to tat_{III} binding to the HIV LTR or which prevent the ability of the tat_{III} protein to trans-activate the HIV LTR (column 12, lines 43-47). The disclosure further points out that compounds that may mitigate the cytopathic effects of HIV include "compounds that inhibit translation and compounds that alter the binding ability of a compound." Indeed, the disclosure discusses at some length a proposed utility for the assay method with respect to this one, specific mechanism of "inhibition" of the tat_{III} protein. The specification, at Col. 12, line 65 to Col. 13, line 43, describes a potential use of the assay method in screening a number of mutant tat_{III} proteins that have ideally been altered to retain their binding ability but would be "deficient in some other trans-activation function." Thus used, the utility of the assay method disclosed in the Haseltine reference would amount to nothing more than a method to assess the competitive binding of the tat_{III} protein relative to these mutationally altered analogs.

18. A number of additional distinctions exist between the disclosure of the Haseltine reference and the Housey patents. Most importantly, the Haseltine reference has not taught a cell which produces or contains a higher, usually non-naturally occurring, level of a given target protein and which exhibits, *solely* as a result of the presence of the target protein, an altered phenotypic characteristic other than the level of the protein in the cell *per se*, which phenotypic characteristic is responsive to inhibitors or activators of the target protein. Rather, in order to reveal this particular biological function of tat_{III}, the assay method disclosed in the Haseltine reference additionally requires, ***at a minimum***:

- 1) the added presence of an HIV LTR promoter containing a functional tat_{III} binding sequence;

- 2) a "selectable" (or reporter) gene of some type, located within a reasonable distance downstream of the HIV LTR to enable its co-expression; and
- 3) the proper functioning of the gene product (protein) encoded by the "selectable" or "reporter" gene referenced in 2) above, in the cell type used for the assay.

19. In summary, therefore, the following multiple distinctions exist between the Haseltine reference and the core enabling technology taught in the Housey patents. These include the following distinctions:

- a. the Haseltine reference does not depict the creation or utilization of cells which have been specifically created to contain (or overproduce via genetic engineering or other methodologies) higher, usually non-naturally occurring, levels of a given target protein, *and which have been subsequently demonstrated to be responsive to inhibitors or activators of substances which directly interact with the target protein;*
- b. the Haseltine reference, unlike the methods of the Housey patents, does not conceive of or provide a cell which overproduces (or otherwise contains) a higher, usually non-naturally occurring level of a given target protein and which, as a result of the increased level of said target protein, exhibits a **responsive** change in a phenotypic characteristic other than the level of said protein in said cell *per se*; the Housey patents explicitly define this concept of a "responsive change" in the specification ('281 patent, Column 10, lines 26-29) as being a change in a phenotypic characteristic which is **responsive** to inhibitors or activators of the given target protein; the Haseltine reference does not test inhibitors or activators of any kind, let alone demonstrate that the method would actually work in this capacity.
- c. in contrast to the Housey patents, the Haseltine reference does not conceive of or demonstrate that cells which contain (or overproduce) higher,

usually non-naturally occurring levels of a given (target) protein may be utilized to identify known or unknown substances which are new (*i.e.* previously undiscovered) inhibitors or activators of the target protein in a cell.

20. Declarant stresses that the ***generalized applicability of the methods upon which the present invention is based*** are amply taught in the disclosures of the Housey patents. These disclosures state unequivocally that the method taught ". . . is clearly generalizable to any gene which is involved in any way in cellular growth control" (column 8, lines 28-30 of the '281 patent); that the method "... establishes, for the first time, the fact that stable overproduction of a protein . . . can result in novel cellular phenotypes . . . which can be directly modulated by chemical agents ***which interact with the protein.***" (emphasis added). No such teachings are conceived, developed or reduced to practice within the disclosure of the Haseltine reference.

21. **I HEREBY DECLARE** that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

FURTHER Declarant sayeth not.

October 22, 1996


Gerard M. Housey, M.D., Ph.D.